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The Susceptibility of Mice to Airborne Infections Following Continuous Exposure to Low Dose Rate γ Radiation (U)

ANNUAL PROGRESS REPORT

by

Myron S. Silverman, Ph.D.

February 1967
(For the period 1 January 1966 to 31 December 1966)

U.S. Army Medical Research and Development Command

Washington, D.C. 20315

Annual Report to the Commission on Radiation Infection
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Contract No. USAMR&DC 8302

U.S. Naval Radiological Defense Laboratory

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SUMMARY

In order to make reasonable estimates of the epidemiological and medical problems which might be encountered by military and civilian populations required to carry out tasks in fall-out areas, information on the susceptibility to infection and the immune response following exposure to low dose-rate radiation is required. The experiments described in this report were designed to supply such information.

LAF_1 mice exposed continuously to Co^{60} γ radiation delivered at 1.0 - 1.5 rad/hour were exposed to an airborne avirulent Listeria monocytogenes or Pasteurella tularensis (LVS) after the accumulation of varying total doses of radiation. Changes in susceptibility of the irradiated mice were determined by comparing the respiratory and subcutaneous LD_{50}'s of the organism for the irradiated mice with those for the non-irradiated mice. The immune response was determined by challenging the survivors of an initial infection with a second infection and determining the mortality.

The effects of continuous low dose-rate γ radiation on the peritoneal mononuclear cell population were determined by estimating the rate of disappearance of the different cell types following exposure to various total doses of radiation.

In addition, the effects of infection with Pasteurella tularensis (LVS) on the protective properties of WR-1607, a radioprotective chemical, were investigated.

Continuous exposure to Co^{60} γ radiation delivered at 1.0-1.5 rad/hour increases the susceptibility of mice to subcutaneous and airborne infections with strains of Listeria monocytogenes and Pasteurella tularensis of relatively low virulence for non-irradiated mice.

Although the irradiated mice were found to be fully as capable as non-irradiated mice of synthesizing antibodies against sheep red blood cells and of rejecting foreign skin grafts, survivors of an initial infection were less resistant to subsequent infection than non-irradiated mice. Data previously reported together with the data from these experiments suggest that macrophages of irradiated animals are readily injured by bacteria or their products. Hence even immune macrophages may be unable to effectively destroy the bacteria.

Small peritoneal lymphocytes disappeared more rapidly during the first week of exposure than during subsequent weeks whereas medium peritoneal lymphocytes and macrophages disappeared at a constant rate during the entire exposure period. Based on the fraction of cells surviving any given exposure, the order of sensitivity to continuous low dose-rate γ radiation was circulating lymphocytes, small peritoneal lymphocytes, medium peritoneal lymphocytes and peritoneal macrophages. This is the same as previously reported for acute whole-body X irradiation.

Preliminary results indicated that the protective effects of WR-1607, a radioprotective chemical, may be abolished by subsequent infection with Pasteurella tularensis.

Based on the data obtained, it would appear that exposure to continuous low dose-rate γ radiation delivered at 1.0-1.5 rad/hour increases the susceptibility to infection. Although the immune response as measured by the ability to synthesize antibodies and to reject foreign skin grafts was not found to be impaired, the irradiated mice were more susceptible to a second infection with either Listeria monocytogenes or a virulent or avirulent Pasteurella tularensis than were non-irradiated mice. The data suggest that this is due to a greater susceptibility of macrophages to the bacteria and/or their toxic products.

FOREWORD

The Investigators are grateful to Colonel Dan Crozier, MC, USA, and Lt Colonel Kenneth Dirks, MC, USA, Walter Reed Medical Unit, Fort Detrick, and Dr. H. T. Eigelsbach, U. S. Army Biological Laboratories, Fort Detrick, for the generous supply of lyophilized Pasteurella tularensis cultures. We are deeply indebted to Mr. Walter Lief, Naval Biological Laboratories, and his staff for carrying out the aerosol challenges with virulent Pasteurella tularensis. The assistance of H.A. Monahan, HM1, USN is gratefully acknowledged.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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1. OBJECTIVES

Although it has been amply demonstrated that exposure to acute whole-body X irradiation lowers the resistance of animals to infection and decreases the ability to develop an immune response, the effects of continuous exposure to low dose-rate radiation are less well documented. It is essential to obtain such information in order to make reasonable estimates on the medical and epidemiological problems that might be encountered by both military and civilian populations who must carry out their tasks in fall-out areas. The objectives of the experiments reported, therefore, are to determine the nature of the injury to the defense systems and the effects on the ability of the irradiated host to develop both humoral and cellular immune responses either following survival from infection or by the administration of vaccines.

2. BACKGROUND

Continuous exposure to Co^{60} γ irradiation delivered at 1.0-1.5 rad per hour has been shown to increase the susceptibility of mice to an airborne infection with Listeria monocytogenes (1). A more rapid proliferation of the organisms occurred in the lungs, liver and spleen of the irradiated mice. In addition, the clearance of the organisms was delayed in surviving irradiated mice. If death occurred, it appeared 2 to 3 days earlier in the irradiated population than it did in the non-irradiated mice and was accompanied by a bacteremia. The importance of these findings in situations where living avirulent strains of organisms are used for immunization has led to an emphasis on this problem using the living avirulent strain of Pasteurella tularensis (LVS) as a model.

Previous work has shown (2, 3) that following total body exposure to a wide range of whole-body X-ray doses (90-1190 R), the macrophage count of the peritoneal cavity either remained unaltered or gradually decreased to approximately 50% of the normal count. The number of lymphocytes, however, rapidly decreased. The decrease in small peritoneal lymphocytes paralleled the decrease in circulating lymphocytes. The decrease in medium sized peritoneal lymphocytes was less and the rate of the decrease was slower.

Despite the apparent lack of effect on the macrophages, as indicated by cell counts, some injury of these cells must have occurred. Contact with Escherichia coli, Staphylococcus aureus or E. coli endotoxin administered intraperitoneally into irradiated mice resulted in a decrease in the number of macrophages (2).

3. APPROACH

The general approach to the studies on susceptibility to infection and the development of immunity consisted of a comparison of the respiratory bacterial LD_{50} of non-irradiated mice with that for mice exposed to varying doses of Cobalt^{60} γ irradiation delivered at 1.0 to 1.5 rad per hour. Immunity was determined by challenging the animals surviving the initial infection with a second respiratory infection and determining the mortality.

The initial studies on the cellular immune response were planned to determine the effects of both acute and continuous low dose-rate radiation on the survival and function of the peritoneal cell populations. However, since in both of the infections being investigated, listeriosis and tularemia, the immune macrophage plays an essential role, and since the respiratory route is used to initiate the infection, the alveolar macrophages have been selected as a more suitable cell population for study.

Details of the methods used have been reported previously (2) and are included in Appendices A and B (4, 5).

4. RESULTS

4.1. Effects of Continuous γ Irradiation of Mice on the Immune Response to Live Listeria monocytogenes.

This portion of the investigation has been completed during the past year. A complete and final report is presented in Appendix A (USNRDL-TR-1072 of 19 September 1966). Briefly, it has been found that 24% of the mice surviving an initial respiratory infection with an LD_{50} of Listeria monocytogenes after exposure to 1700-2200 rad γ irradiation resisted a second challenge with 5 LD_{50}'s of the organism. If the animals were exposed to 2800-3000 rad prior to the initial infection, only 54% of the surviving animals developed sufficient immunity to withstand the second respiratory infection. Essentially all of the non-irradiated mice survived both the first and second infections. However, immunity was of short duration. If the second challenge was given 4 weeks after the first infection rather than 2 weeks, only 10% of the irradiated animals survived compared to 75% of the non-irradiated.

Clearance of the organisms from the lungs, liver and spleen was rapid in the non-irradiated immune group. By the fourth day after challenge few organisms could be isolated. If the mice had been irradiated prior to immunization, clearance was delayed in some animals, but not in others. Large numbers of the infecting agent could be isolated from the organs of both the irradiated and non-irradiated non-immune groups.

4.2. Effects of Continuous γ Irradiation of Mice on the Susceptibility and Immune Response to Respiratory Infection with Pasteurella tularensis.

The proposed use of a living avirulent strain of Pasteurella tularensis (LVS) for immunization of military and civilian populations has posed the question of whether the general increased susceptibility to infection following exposure to either acute whole-body radiation or continuous exposure to low dose-rate radiation renders the use of such a vaccine inadvisable in situations involving radiation exposure. In addition, the question of whether the irradiated individual will develop an immune response to the vaccine must be determined. For these reasons, attention has been focused on the response of irradiated mice to this vaccine.

The methods of radiation exposure used were the same as those described in the earlier report (2). Both infection and immunization were induced by either subcutaneous injection of LVS or by exposure to an aerosol of the organisms (2). The mice used were (C57L x A) F_1, (LAF_1), male mice. The challenge with both LVS and the virulent SCHU-5 strain of Pasteurella tularensis infections were given by the respiratory route.

LVS was obtained as lyophilized cultures from Fort Detrick, Frederick, Maryland. After suspending the organisms in a gelatin-saline solution, they were inoculated on SB Agar (6) or Cystine Heart Agar (Difco) enriched with hemoglobin (Difco). In some cases, Glucose Cystine Blood Agar (Baltimore Biological Labs.) enriched with Cleland's reagent (Dithiothreitol) was used. After incubation for 72-96 hours at 37°C, the organisms were washed from the agar plates, concentrated by centrifugation at 5000 RPM for 20 minutes and resuspended in gelatin-saline to give the desired concentration.

The susceptibility of irradiated mice following both subcutaneous injection or exposure to an aerosol was compared to that of non-irradiated animals of the same age group.

Table I indicates that after exposure of mice to accumulated radiation doses ranging from 500-2500 rads delivered at 1.4 rad per hour, an increase in susceptibility of mice to a subcutaneous infection occurred. Although the present data is somewhat erratic, it is obvious that in this infection, as is true with other infections studied, susceptibility increased as the total accumulated radiation dose increased. Non-irradiated mice proved to be quite resistant to subcutaneous injections with LVS. No deaths were observed in mice receiving doses ranging from 7.0 to 4.0×10^6. Twenty-eight percent of the mice (2 out of 7) injected with 4.0×10^7 organisms died and 33% (3 out of 10) of the animals died after the administration of 4.0×10^9 cells.

TABLE I

SUSCEPTIBILITY OF CONTINUOUSLY IRRADIATED LAF_1 MICE TO SUBCUTANEOUS INFECTIONS OF PASTEURELLA TULARENSIS (LVS)

No. of organisms injected*	Accumulated Radiation Doses (rads)							
	100-500		600-1200		1200-1800		2000-2500	
	Dead / Total	% Dead	Dead / Total	% Dead	Dead / Total	% Dead	Dead / Total	% Dead
7×10^0	6/20	30	---	--	21/30	70	29/30	97
9.8×10^0	1/10	10	---	--	3/10	30	7/10	70
9.8×10^1	3/49	6	6/38	16	24/31	77	20/20	100
8.4×10^2	4/39	11	1/10	10	15/50	30	43/51	84
5.4×10^3	3/30	10	15/50	30	33/40	82	42/50	84
5.8×10^4	4/9	44	43/51	84	18/19	95	19/19	100

* All non-irradiated mice survived subcutaneous injections of 4×10^6 organisms.

The suceptibility of non-irradiated mice to a respiratory infection with Pasteurella tularensis (LVS) was found to be greater than that to subcutaneous infections. The LD_{50} calculated by the method of Litchfield and Wilcoxon (7) from the data in Table II, was 1.5×10^3 (95% confidence limits were 9.4×10^2 - 2.4×10^3). Because of the low LD_{50} in non-irradiated mice, it was difficult to accurately determine the respiratory LD_{50} for irradiated mice. Table III suggests that following γ irradiation exposure totaling 1500 rad and 2500 rad, the LD_{50} of LVS may be about 1×10^2 cells.

Mice surviving the initial subcutaneous infections were subjected to a respiratory challenge with the virulent strain of Pasteurella tularensis, SCHU-5, 30 days after the initial exposure in order to determine the immune response to LVS. Survival of the mice was used as the index of immunity. Table IV indicates that in all of the irradiated groups the development of an immune response was decreased, compared with the non-irradiated immunized animals. The respiratory LD_{100} of the SCHU-5 strain was less than 50 organisms for both non-irradiated and irradiated non-immune mice. In addition, it was found that greater than 1×10^3 organisms resulted in 100% mortality in immunized mice whether irradiated or not.

Because of the poor survival following immunization with LVS by the respiratory route, data on the immune response to a challenge infection with SCHU-5 are scanty. Those animals challenged with 1×10^2 organisms gave evidence of good protection since all survived. However, if the challenge dose was increased to 1×10^3, neither irradiated nor non-irradiated immune mice survived.

In view of the poor immune response of both irradiated and non-irradiated mice to a single immunization by either the subcutaneous or respiratory routes, an investigation of the immune response to a combination of the two routes was carried out. Mice were first immunized by subcutaneous injection followed 2 weeks later by exposure to an aerosol of LVS. Three to 4 weeks after the aerosol exposure, the mice were challenged with a respiratory infection of virulent SCHU-5. The subcutaneous immunizing doses of LVS were 1.6×10^1 or 3.4×10^1 organisms. The respiratory immunizing doses were 6.8×10^3 or 9.0×10^3 and the challenge doses were either 5×10^1 or 1.8×10^2 SCHU-5 cells. All of the non-irradiated non-immune mice died after receiving the SCHU-5 challenge while the non-irradiated immune mice all survived. There were no deaths in the groups of mice immunized after receiving Co^{60} γ radiation in doses ranging from 100 rad through 1500 rad and challenged with either 5×10^1 or 1.8×10^2 virulent SCHU-5, nor were there any deaths resulting from the immunization procedures.

TABLE II

SUSCEPTIBILITY OF NON-IRRADIATED LAF_1 MICE TO A RESPIRATORY INFECTION WITH PASTEURELLA TULARENSIS (LVS)

Inhaled Dose of organisms	Dead / Total	Percent Dead
1.1×10^1	0/35	0
9.4×10^1	0/20	0
2.6×10^2	3/35	8
3.6×10^3	25/33	76
3.8×10^3	15/19	79
1.6×10^4	33/33	100

TABLE III

SUSCEPTIBILITY OF LAF_1 MICE TO RESPIRATORY INFECTION WITH PASTEURELLA TULARENSIS (LVS)* FOLLOWING CONTINUOUS EXPOSURE TO Co^{60} γ IRRADIATION

Total γ Irradiation (Rad)	Dead / Total	Percent Dead
500	3/20	15
1500	10/20	50
2500	11/20	55
0	0/20	0

* The challenge dose was 9.4×10^1 cells.

TABLE IV

SURVIVAL OF IRRADIATED LAF_1 MICE TO RESPIRATORY INFECTION WITH SCHU-5 STRAIN OF *PAST. TULARENSIS* AFTER SUBCUTANEOUS IMMUNIZATION WITH LVS

EXPERIMENT A

Total Radiation Dose (rad)	Immunization Dose (LVS):	9.8×10^0		9.8×10^1		4.2×10^2		1.3×10^3	
	Challenge Dose (SCHU-5):	5.5×10^2		5.5×10^2		7.9×10^2		2.0×10^2	
		Survivors / Total	%	Survivors / Total	%	Survivors / Total	%	Survivors / Total	%
500		3/9	33	1/6	16	12/28	44	17/19	90
1500		4/7	57	0/3	0	5/18	28	---	--
2500		0/3	0	---	--	0/8	0	---	--
Non-Radiated Immune		7/9	88	6/8	75	7/20	85	9/9	100
Non-Irradiated Non-Immune		0/10	0	---	--	0/18	0	0/10	0

EXPERIMENT B

Total Radiation Dose (rad)	Immunization Dose (LVS):	5.8×10^4		1.8×10^3		None	
	Challenge Dose (SCHU-5):	1.4×10^2		1.5×10^2		1.3×10^2	
		Survivors / Total	%	Survivors / Total	%	Survivors / Total	%
750		4/9	45				
1200		5/8	62				
1400				4/7	57		
2400				3/8	37		
None				7/8	88		
None						0/10	0

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4.3 Effects of Continuous γ Irradiation on the Formation of Circulating Antibodies and Rejection of Allogeneic Skin Grafts.

Although immunity to infection is impaired by continuous exposure to low dose-rate γ irradiation, Bensted (8) has reported that a total dose of 1400 rad delivered at 50 rad/day of continuous exposure did not decrease the hemagglutinin formation to sheep red blood cells in mice. Previous work done in our laboratory (9) also indicated no decrease in the response to sheep red blood cells of mice, exposed to either 1200 or 2200 rad. In addition, the time of rejection of foreign skin grafts was not prolonged by exposure to these doses. During the past year, this work has extended to test the response of mice exposed to higher doses of low dose-rate γ radiation. Briefly, it can be stated that neither 3200 or 3800 rad total cumulative dose of Co^{60} γ irradiation delivered at 1.0-1.5 rad/hour effects the ability of LAF_1 mice to produce hemagglutinins against sheep red blood cells. Neither the peak titers nor the time required to reach peak titer were significantly different from those obtained with non-irradiated mice. LAF_1 mice exposed to 3200, 4800 or 5500 rad γ irradiation were also found to be as fully capable of rejecting BALB/c mouse skin grafts as were their corresponding non-irradiated controls. No significant difference in the time of rejection was obtained. This data is of importance in determining the nature of impaired resistance of irradiated immunized mice to infection.

4.4. Effects of Continuous Low-level γ Irradiation on Circulating and Peritoneal Mononuclear Leucocytes of Mice.

A detailed report on this phase is included as Appendix B (USNRDL-TR-1085 of 20 October 1965). In summary it was found that if LAF_1 mice were exposed continuously to Co^{60} γ irradiation at a dose rate of 1.4 rad per hour, the number of lymphocytes in the circulating blood fell sharply during the first week of exposure (190 rad) and decreased thereafter at a very gradual but statistically significant rate for the duration of the experiment (15 weeks, 3450 rad). The disappearance of small lymphocytes (6 μ in diameter) from the peritoneal cavity was also more rapid during the first week of irradiation than during subsequent weeks. Medium peritoneal lymphocytes (8-10 μ in diameter) and peritoneal macrophages disappeared at constant rates over the entire observation period. After the first week of exposure, the disappearance rates of small and medium peritoneal lymphocytes were identical. This rate was greater than that for peritoneal macrophages and that for circulating lymphocytes.

Based on the fraction of cells surviving any given exposure, the mononuclear leucocytes may be arranged in the following order of decreasing sensitivity to continuous low-dose rate γ irradiation: circulating lymphocytes, small peritoneal lymphocytes, medium peritoneal

lymphocytes, peritoneal macrophages. This order is the same as that after acute exposure to X rays.

4.5. Effects of Radioprotective Chemical WR-1607 on the Susceptibility of Irradiated and Non-irradiated Mice to Infection.

One of the more promising radioprotective chemicals, WR-1607, was tested for its effects on susceptibility to infection in irradiated and non-irradiated mice.

WR-1607 was prepared as a suspension in saline and 0.35 mg was injected intraperitoneally into LAF_1 mice immediately prior to exposure to 800 rad whole-body X radiation. At various times after drug treatment and irradiation, the mice received a subcutaneous injection of Pasteurella tularensis (LVS). The data obtained thus far are tabulated in Table V.

It can be seen that although WR-1607 markedly decreased the mortality due to radiation, the subsequent injection of Pasteurella tularensis (LVS) completely abolished the protective effect of the drug. The data suggest that death of the irradiated mice was due primarily to the infection, since LVS + WR-1607 in non-irradiated animals had no significant effect.

5. DISCUSSION

5.1. Effects of Continuous γ Irradiation of Mice on Susceptibility to Infection and the Immune Response.

Continuous exposure to low dose-rate γ irradiation has been shown to increase the susceptibility of mice to respiratory infections with both Listeria monocytogenes and Pasteurella tularensis (LVS). The latter organism, although of low virulence for the mouse when injected subcutaneously has been found to be virulent when administered via the respiratory route. This verifies similar observations made in other laboratories.

Development of immunity in irradiated mice in both listeriosis and tularemia was impaired when tested by challenge. However, the immune response when tested by antibody formation against sheep red blood cells or rejection of allogeneic skin grafts was not found to differ from that of non-irradiated animals. Both of the infections studied belong to the type in which immunity is dependent more upon the development of "immune" macrophages rather than the formation of circulating antibodies. Although the role of macrophages in rejection of skin grafts is, perhaps, still open to question, the development of a

TABLE V

EFFECTS OF RADIOPROTECTIVE CHEMICAL, WR-1607,
ON THE SUSCEPTIBILITY OF IRRADIATED AND NON-IRRADIATED MICE
TO INFECTION WITH PASTEURELLA TULARENSIS (LVS)

Treatment	Percent Mortality			
	Time (hours) Post-Irradiation of Injection of LVS			
	1	24	48	72
800 rad only	100	100	100	100
1607 + 800 rad	10	0	0	29
1607 + 800 rad + LVS	100	100	100	100
800 rad + LVS	90	100	100	100
1607 + LVS	30	0	0	0
LVS only	0	0	0	0
No. of LVS injected	1.9×10^3 - 1.3×10^5	1.1×10^4	5.6×10^3	8.2×10^2

delayed hypersensitivity reaction following tissue rejection suggests that sensitized macrophages do play an important role. If this is so, our data would indicate that low dose γ irradiation probably does not affect the sensitization of the cells. This leads to the conclusion that the decreased resistance of survivors of a primary infection to subsequent infection must be due to causes other than the inability to give rise to an immune response.

In addition, the data obtained from the studies on peritoneal cell populations (see Appendix B) indicates that macrophages are comparatively resistant to low dose-rate γ irradiation. A decrease in the number of macrophages does occur slowly, if the total radiation dose is sufficiently high. However, it is doubtful whether the decreased immune response is due to the decrease in macrophages.

Data presented in our previous report (2) indicated that peritoneal macrophages from acute whole body X-irradiated mice were injured by both Escherichia coli and Staphylococcus aureus and by the E. coli endotoxin.

Based on all of these results one may postulate, then, that the greater sensitivity of macrophages from irradiated mice to bacteria or bacterial products is sufficient to overcome the immunity. The bacteria (or their products) may damage the cells and allow the bacteria to proliferate and be disseminated. This in turn would eventually give rise to a generalized infection and death. The complete protection obtained with a combined subcutaneous and aerosol immunization suggests that if a high degree of immunity can be elicited the cells can resist the toxic effects of the organisms and function in a normal manner. This hypothesis must still be tested.

5.2. Effects of Radioprotective Chemical WR-1607 on the Susceptibility of Irradiated and Non-irradiated Mice to Infection.

The preliminary data obtained indicate that WR-1607 does not decrease resistance of irradiated or non-irradiated mice to infection, but rather that the infection abolishes the effectiveness of the drug in protecting against lethal irradiation. Whether this is true of infections other than tularemia must still be determined.

It would appear from the data available that WR-1607 does not protect against irradiation by stimulating functional recovery of the reticulo-endothelial system, since administration of the chemical does not give rise to protection of the host's cellular anti-bacterial defense system. However, a definite conclusion must await data from animals challenged after longer intervals.

6. CONCLUSIONS

a. Continuous exposure to low dose-rate γ irradiation delivered at 1.0 to 1.5 rad per hour increases the susceptibility of mice to airborne infections with strains of Listeria monocytogenes and Pasteurella tularensis of relatively low virulence for non-irradiated mice.

b. Although these irradiated animals are capable of forming circulating antibodies and of rejecting foreign skin grafts as efficiently as non-irradiated mice, survivors of an initial infection are less resistant to subsequent infection than were non-irradiated controls.

c. The data suggest that macrophages of irradiated animals are readily injured by bacteria and/or their products. Hence even immune phagocytic cells may be unable to effectively destroy the invading organisms.

d. Preliminary results indicate that the protective effects of a radioprotective chemical, WR-1607, may be prevented by infection. The data suggest that the protective effect is not due to protection of the host's antibacterial defense mechanisms.

7. RECOMMENDATIONS

a. The results obtained in rodents should be extended to determine the effects of continuous exposure to low dose-rate radiation on infection and immunity in primates.

b. As an additional model, an infection, in which circulating antibodies play the important role in the immune response, should be studied.

c. Investigation on the effects of bacteria on immune and non-immune macrophages from irradiated animals should be continued and extended.

d. Based on the data obtained to date, the use of living avirulent strains of bacteria for immunization of individuals continuously exposed to low dose-rate ionizing radiation is not recommended.

e. Prospective radioprotective chemicals should be tested for their effects on susceptibility to infection.

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D I S T R I B U T I O N L I S T

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A P P E N D I X A

USNRDL-TR-1072 of 18 September 1966

EFFECTS OF CONTINUOUS IRRADIATION OF MICE ON THE IMMUNE RESPONSE TO LIVE *LISTERIA MONOCYTOGENES* VACCINE

by

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USNRDL-TR-1072
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ADMINISTRATIVE INFORMATION

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ABSTRACT

Mice exposed continuously to radiation delivered at 1.0-1.5 rad/hour were exposed to a respiratory infection with a midlethal dose of a live avirulent strain of Listeria monocytogenes immediately after accumulating either 1700-2200 rad or 2800-3000 rads. The surviving mice were challenged two weeks later with a second aerosol containing the organism in order to determine their immune state. All of the non-irradiated mice exposed to the two aerosol infections survived while 24% of the 1700-2200 rad irradiated mice and 54% of the 2800-3000 rad groups succumbed to the second infection. If the irradiated mice were immunized with two aerosol exposures at a two week interval both the irradiated (2200 rad) and the non-irradiated animals survived. Immunity following a single exposure was of short duration. If the challenge was postponed until 4 weeks after the immunizing exposure, 90% of the irradiated mice died.

Clearance of L. monocytogenes from the lungs, liver and spleen was rapid in the non-irradiated immune group. By the fourth day after challenge, few organisms could be isolated. If the mice were irradiated prior to immunization, clearance was delayed. Bacteria could still be found in all organs. Large numbers of bacteria could be isolated from both groups of non-immune mice.

NON-TECHNICAL SUMMARY

The Problem

Increasing interest is being focused on the possible use of airborne avirulent bacterial and viral strains as a possible means of immunization against a subsequent infection with virulent strains. This method of immunization is applicable in both clinical situations and in protection against biological warfare. Additional interest has been expressed regarding the possibility that exposure to low dose rate gamma radiation (such as might be encountered in a radiation fallout field) may decrease the individual's resistance to a live avirulent immunizing agent to the extent that serious illness or death might result from the immunization itself. Also the question has been raised as to whether a person's ability to acquire immunity might be impaired by exposure to low dose rate gamma radiation.

The Findings

These studies have shown that chronically irradiated mice are more susceptible to an immunizing exposure of Listeria monocytogenes than non-irradiated animals. In addition, the ability of the surviving irradiated mice to acquire immunity within two weeks appeared to be impaired. The immune response decreased as the total dose of radiation increased, as indicated by a smaller number of survivors

following respiratory challenge with a lethal dose of organisms. When the interval between aerosol immunization and challenge was increased to four weeks, a decrease in percent survivors was noted in both non-irradiated immunized and irradiated (2200 rad) immunized mice. It was more pronounced however, in the irradiated immunized group. Two immunizing exposures resulted in essentially 100% protection in both irradiated and non-irradiated mice.

Studies on the growth of the organisms in the irradiated animals indicated that both the irradiated and non-irradiated immune mice were able to destroy the invading bacteria more rapidly than the non-immune.

INTRODUCTION

It was shown previously (1) that exposure of mice to chronic gamma radiation delivered at 1.0-1.5 rad/hr resulted in a marked increase in susceptibility to airborne infection with Listeria monocytogenes. As the cumulative radiation dose increased the susceptibility to infection increased so that mice receiving a total of 2500 rad over a two month period were over 33 times as susceptible to fatal infection as those receiving no radiation.

The resistance of animals to L. monocytogenes can be enhanced by immunization with sublethal doses of the virulent live organism (2-7). Since protection is not afforded by passive immunization with antiserum (5,8,9) and since, as Seeliger (10) points out, no relationship exists between circulating antibody titers and the severity of infection or degree of immunity in humans, it has been concluded that resistance to L. monocytogenes is not mediated by by humoral factors. Thus, as in tuberculosis, brucellosis and tularemia, it has been claimed that an alternative mechanism, probably mediated by cells, plays a role in acquired resistance to Listeriosis. Related studies (12,13) have supported this concept of acquired cellular immunity, but have emphasized that the cellular resistance is non-specific in nature.

The studies to be reported here deal with the effects of prior exposure of mice to chronic gamma radiation on the development of acquired immunity following airborne challenge with L. monocytogenes.

MATERIALS AND METHODS

Mice *

Equal numbers of male and female LAF_1 (C57L ♀ x A/He ♂) mice from our laboratory colony were used in the experiment. Mice were 12 to 16 weeks old at the time of exposure to bacterial aerosols.

Irradiation of Mice

Mice were continuously exposed to γ radiation from a Co^{60} source at a dose rate of 1.0-1.5 rad/hour until the desired accumulated doses were obtained. Plastic mouse cages housing 10 mice each were placed on curved wooden racks so that the center of each was equidistant from the Co^{60} pellet. Initial studies employed a 2.5 curie Co^{60} source. Dose measurements were made with a Philips standard dosimeter. Later studies were done with a lead shielded 10.8 curie Co^{60} source. Dose measurements were made with TLD System Dosimeters. The Co^{60} source was in continuous operation except for

* In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

1 hour per week when the cages were changed. Fresh food pellets and water were also supplied at this time. No deaths occurred among the mice during the radiation exposure period or among animals held as long as six weeks after removal from the Co^{60} source.

Listeria monocytogenes

Media and growth conditions used in the cultivation of L. monocytogenes have been previously described (1).

Exposure of Mice to Bacterial Aerosol

Mice were infected by exposure to aerosols of L. monocytogenes in a modified Henderson apparatus (14). Irradiated mice were exposed within 2 hours after removal from the Co^{60} source. The aerosol was sampled with impingers simultaneously with exposure of the animals. Calculations of the dose inhaled by the experimental animals were made from the data obtained on the concentration of cells collected in the impinger fluid and from the respiratory rate and volume of the animal according to Guyton's formula (15).

Exposed animals were observed for deaths. Immunity was measured by challenging the survivors to a second respiratory infection 2 to 4 weeks later using a dose of organisms that was normally lethal for non-irradiated mice. Survival following the second exposure was used as an index of immunity. Animals were observed for 30 days after the last aerosol exposure. Most mice that succumbed were autopsied and

examined for gross pathological changes. Bacteriological studies showed that, with very rare exceptions, L. monocytogenes could readily be isolated from the lungs, livers and spleens of the dead mice.

Bacterial Enumeration in Lung, Liver and Spleen

Organs were removed, homogenized, and aliquots were plated on tryptose agar as previously described (1). Results were expressed as the number of viable organisms per organ.

RESULTS

Initial experiments were designed to determine if non-irradiated and irradiated mice could be immunized by means of an aerosol against a subsequent lethal aerosol challenge of L. monocytogenes. Both non-irradiated and irradiated mice were initially exposed to the same immunizing doses of the airborne Listeria. Since irradiated mice are more susceptible to an initial airborne infection (1), the immunizing exposure was adjusted so that fewer than 50% of the irradiated mice would die after primary exposure. This was a non-lethal dose for a non-irradiated population. Two weeks after exposure to the immunizing aerosol of Listeria the surviving mice were challenged with approximately 5 LD_{50}'s of the microorganism for a non-irradiated, non-immunized population. This dose was sufficient to kill all

irradiated non-immune mice and all but a few of the control group.

From the data presented in Table I, it was evident that exposure to continuous low dose rate gamma radiation resulted in some decrease in the immune response. Although essentially all the non-irradiated immune mice survived, 24% of all the mice immunized after exposure to 1700-2200 rad died, while 54% of the mice accumulating 2800-3000 rad succumbed to the challenge dose of L. monocytogenes. The majority of non-irradiated mice surviving the initial immunizing dose of $2.9\text{-}4.2 \times 10^4$ organisms manifested physical signs of infection after the challenge dose of L. monocytogenes. However, all except 2 of the 58 mice recovered.

A comparison of the effectiveness of two immunizing aerosol exposures on the ability of non-irradiated and irradiated mice to survive a challenge dose of 1.7×10^6 Listeria is presented in Table II. The primary immunizing aerosol was given four weeks before challenge and the second immunizing aerosol was given two weeks in advance of challenge. Mice were removed from the Co^{60} source two hours before the primary immunization and were not subjected to further radiation. The percent survival following challenge with 1.7×10^6 cells was comparable in non-irradiated and irradiated (2200 rad) mice when both groups had received two immunizing aerosols. In the group of mice which had only a single immunizing dose four weeks

TABLE I

SURVIVAL OF MICE IMMUNIZED BY AEROSOL EXPOSURE TO *LISTERIA MONOCYTOGENES*.

Group	Immunizing Dose	Challenge Dose	Dead Total	% Dead
Irradiated Immune				
1700 rad	4.2×10^4	3.7×10^6	8/40	20
1900 rad	2.9×10^4	2.6×10^6	11/37	30
2200 rad	3.5×10^4	2.6×10^6	6/28	21
2600 rad	1.9×10^4	1.7×10^6	18/28	64
2677 rad	7.8×10^2	7.6×10^6	7/17	41
2900 rad	1.3×10^5	5.5×10^6	9/19	47
2986 rad	5.4×10^4	6.3×10^6	11/19	58
Irradiated Non-Immune				
2200 rad	---	2.6×10^6	20/20	100
2677 rad	---	7.6×10^6	18/19	95
2900 rad	---	5.5×10^6	8/8	100
3000 rad	---	6.3×10^6	10/10	100
Non-Irradiated Immune				
	4.2×10^4	3.7×10^6	2/40	5
	2.9×10^4	2.6×10^6	0/9	0
	7.8×10^2	7.6×10^6	0/19	0
	1.3×10^5	5.5×10^6	0/20	0
	5.4×10^4	6.3×10^6	0/20	0
Non-Irradiated Non-Immune				
	---	1.7×10^6	90/98	86
	---	2.6×10^6	52/60	87
	---	3.0×10^6	17/20	85
	---	3.7×10^6	55/62	89
	---	4.2×10^6	30/31	97
	---	5.5×10^6	8/10	80
	---	6.3×10^6	7/10	70
	---	7.6×10^6	10/10	100

TABLE II

SURVIVAL OF CONTINUOUSLY γ-IRRADIATED MICE TO *LISTERIA MONOCYTOGENES*

FOLLOWING PRIMARY AND SECONDARY AIRBORNE IMMUNIZATION

Group	Primary Immunizing Dose	Secondary Immunizing Dose	Challenge Dose	Survival Total	% Survival
Irradiated *	2.6×10^4	1.6×10^4	1.7×10^6	17/18	95
	2.6×10^4	None	1.7×10^6	2/20	10
	None	None	1.7×10^6	0/10	0
Non-Irradiated	2.6×10^4	1.6×10^4	1.7×10^6	20/20	100
	2.6×10^4	None	1.7×10^6	15/20	75
	None	None	1.7×10^6	2/20	20

* 2200 rad γ Radiation at 1.5 rad/hour.

before challenge, 75% of the non-irradiated mice and only 10% of the irradiated mice survived. None of the irradiated non-immunized mice survived, although 20% of the non-irradiated non-immunized animals were able to do so.

Since it had been determined that both non-irradiated and irradiated mice could acquire immunity to L. monocytogenes following aerosol immunization, providing the total radiation exposure did not exceed approximately 2000 rad, an additional parameter was studied to supplement these findings. The distribution of Listeria was followed in the lung, liver and spleen of animals from the four groups over a four day period following a challenge of 1.5×10^6 bacteria, in order to determine the clearance of the organisms by these organs (Table III).

As expected, extensive bacterial proliferation was found in the organs of non-immune groups of mice following the challenge. With the exception of initial clearance by the lungs at four hours post infection, between 10^5 and 10^8 bacteria were found in all organs on the second and fourth days following aerosol exposure. On the other hand, although Listeria did spread to a slight extent from the lungs to the liver and possibly the spleen of non-irradiated immunized mice, it was quite evident that by the second day significant suppression of

TABLE III

THE RECOVERY OF LISTERIA MONOCYTOGENES FROM IRRADIATED AND NON-IRRADIATED, IMMUNE MICE

Group	Survivors Total	% Survivors	Colony Count Per Organ							
			Zero Hour Lung	4 Hours Lung	Day 2 Lung	Day 2 Liver	Day 2 Spleen	Day 4 Lung	Day 4 Liver	Day 4 Spleen
Irradiated immunized [a]	6/21	76	1.5×10^5	1.9×10^4	9.6×10^4	1.9×10^3	5.0×10^3	3.0×10^1	1.2×10^4	$< 2.0 \times 10^1$
			1.0×10^5	1.2×10^4	5.2×10^5	2.5×10^6	5.0×10^3	1.6×10^7	1.1×10^8	4.5×10^5
			1.3×10^5	3.0×10^4	3.9×10^4	2.4×10^4	2.2×10^3	8.4×10^2	4.2×10^2	$< 2.0 \times 10^1$
Irradiated non-immunized [b]	0/25	0	6.8×10^4	2.6×10^4	4.9×10^6	8.8×10^6	1.8×10^5	1.1×10^8	2.4×10^8	1.2×10^7
Non-Irradiated immunized [a]	16/16	100	1.6×10^5	3.4×10^4	6.0×10^2	7.8×10^3	$< 2.0 \times 10^2$	$< 3.0 \times 10^1$	6.0×10^1	$< 2.0 \times 10^1$
			9.0×10^4	contaminated	$< 3.0 \times 10^2$	$< 6.0 \times 10^2$	$< 2.0 \times 10^2$	$< 3.0 \times 10^1$	$< 6.0 \times 10^1$	$< 2.0 \times 10^1$
			1.2×10^5	1.5×10^4	3.0×10^2	1.0×10^4	$< 2.0 \times 10^2$	$< 3.0 \times 10^1$	$< 6.0 \times 10^1$	$< 2.0 \times 10^1$
Non-Irradiated non-immunized [a]	2/18	11	1.5×10^5	3.7×10^4	4.0×10^6	2.0×10^6	1.7×10^5	7.0×10^6	6.2×10^7	1.8×10^5
			1.4×10^5	2.6×10^4	3.3×10^6	4.5×10^6	3.1×10^5	1.2×10^8	1.3×10^8	3.3×10^6
			1.9×10^5	7.3×10^4	4.7×10^6	1.0×10^7	1.6×10^5	1.8×10^7	8.3×10^7	2.5×10^5

[a] Inhaled challenge dose = 1.5×10^6 Listeria Monocytogenes.

[b] Inhaled challenge dose = 8.0×10^5 Listeria Monocytogenes.

bacterial growth had occurred and by the fourth day no detectable bacteria were present in these organs.

Distribution of Listeria in the organs of irradiated immunized mice showed a variable response following the aerosol challenge. The number of microorganisms recovered was less than that encountered in both non-immunized groups of mice, but more than that observed in non-irradiated immunized mice. By the fourth day there was a wide variation in the numbers of recoverable bacteria. Suppression of bacterial growth was observed in the lungs, liver and spleen of two mice, whereas large numbers of Listeria were recovered from the third mouse.

DISCUSSION

These studies have shown that both non-irradiated mice and mice exposed to continous low dose rate γ radiation can be immunized by the respiratory route against a subsequent challenge of a normally lethal dose of airborne Listeria, providing the total dose of radiation is not too high.

Even though the immunizing aerosol dose of Listeria ($1.2\text{-}4.2 \times 10^4$ cells) was adjusted so that no deaths occurred in a non-irradiated population, the increased susceptibility of mice, exposed to Co^{60} γ radiation (1) caused deaths in some of the mice

after exposure to the immunizing dose of bacteria. From the data, it appeared that a difference existed in the response of non-irradiated immunized and irradiated immunized mice challenged with a comparable dose of the Listeria aerosol. This difference in percent survival, although apparent, may not have existed had the challenge aerosol doses for the two groups been based on a challenge dose consisting of a comparable multiple of the bacterial LD_{50} for each group. Mice which had been irradiated, immunized and challenged were more susceptible than were non-irradiated immune mice exposed to the same bacterial challenge. In view of these facts, the most valid comparisons are those between the immune and non-immune irradiated mice and between the immune and non-immune non-irradiated animals rather than between irradiated and non-irradiated groups. On the basis of these comparisons one can conclude from the available data that both immunized populations demonstrated a greater resistance to the high dose aerosol challenge than did the non-immune animals. However, the lower number of survivors among irradiated immunized mice indicates that impairment of their ability to acquire immunity had occurred.

Experiments in which non-irradiated and irradiated mice received two immunizing bacterial aerosol exposures at 14 day intervals before challenge indicated that both groups were quite resistant when challenged with an identical aerosol dose. However, irradiated mice immunized

with a single dose and held four weeks before challenge showed a very low percent survival compared to non-irradiated mice similarly handled. This undoubtedly was a reflection of both the short duration of immunity and remaining injury resulting from radiation. Non-irradiated mice challenged four weeks after a single immunizing aerosol, although more resistant than irradiated mice similarly treated, showed less resistance to the challenge than non-irradiated mice immunized at 28 and 14 days before challenge. These findings are in accordance with those of Mackaness (11) who suggested that immunity to listeriosis in the mouse, although strong, is of relatively short duration following immunization.

Comparison of the data obtained on bacterial numbers in the lung, liver and spleen homogenates of non-irradiated and irradiated groups of mice following challenge proved quite interesting in light of our previous data on percent survival following aerosol infection with large numbers of microorganisms. As expected, extensive bacterial growth was found in the organs of non-immunized irradiated and non-irradiated mice on days 2 and 4 following challenge. However, on the same days, bacterial counts from the organs of non-irradiated immunized mice indicated that the organism had failed to grow in the tissues of these mice. These findings are in agreement with the thesis that the antibacterial mechanism developed during the primary

infection is retained after recovery from the primary infection (11). It has been our experience that no bacteria can be found in the organs of surviving irradiated or non-irradiated mice 14 days after a primary infection. Thus, any bacteria recovered from the organs of mice challenged at this time can be attributed only to those inhaled at challenge. The clearance of bacteria from the organs of two irradiated immunized mice and the bacterial growth in another was undoubtedly a reflection of the variable survival rate (76 %) observed in this group following aerosol challenge.

Bensted (16) has reported that mice exposed to 1400 rad of γ radiation delivered at 50 rad/day were as fully capable of producing hemagglutinins to sheep red blood cells as were non-irradiated controls. Silverman (17) also found no inhibition of antibody formation to sheep red blood cells in mice receiving a total of 1200 or 2200 rad delivered at 36 rad/day. In addition, mice similarly irradiated were able to reject allogenic skin grafts as readily as non-irradiated controls. The inhibition of the immune response to Listeria monocytogenes may appear to be in contradiction to these results. The determination of an immune response to bacterial infection by challenge, however, is a measure not only of the response of the host to the immunization, but also its interaction with the challenge organism. We have shown previously (1) that resistance of continuously irradiated mice to

infection with this organism is considerably reduced. This is further borne out by the experiments which showed that the organism can proliferate in the lungs, liver and spleen of some of the irradiated immune animals. Thus, it might be expected that in some of these immune mice the balance between the immune response and the irradiation injury would be tipped in favor of the invading organism. Presumably, if the total radiation dose received was increased further, conditions would be even more advantageous to the organism.

If, as Mackaness states (11), immunity to Listeriosis in the mouse is a cellular response due to the increase capacity of the macrophage to resist intracellular growth of the organism, irradiation with a sufficiently high dose might be expected to prevent the development of the immune response. Donaldson, et al., (18) and Nelson and Becker (19) have shown that these cells lose their bactericidal properties following acute radiation in the mid-lethal range. Kornfeld and Greenman (20) have found a reduction in the numbers of peritoneal macrophages in mice exposed to continuous γ radiation delivered at about 1.4 rad/hour. Neither the phagocytic function nor bactericidal properties of the macrophages from the continuously irradiated mice were tested. However, the results of the experiments presented in this report would suggest a functional impairment.

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EFFECTS OF CONTINUOUS LOW-LEVEL GAMMA IRRADIATION ON CIRCULATING AND PERITONEAL MONONUCLEAR LEUCOCYTES OF MICE

by

L. Kornfeld

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USNRDL-TR-1085
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ABSTRACT

LAF_1 mice were exposed continuously to Co^{60} gamma radiations at a dose rate of 1.4 rads per hour. The number of lymphocytes in the circulating blood fell sharply during the first week of exposure (190 rads) and decreased thereafter at a very gradual but statistically significant rate for the duration of the experiment (15 weeks, 3450 rads). The disappearance of small lymphocytes (6 μ in diameter) from the peritoneal cavity was also more rapid during the first week of irradiation than during subsequent weeks. Medium peritoneal lymphocytes (8-10 μ in diameter) and peritoneal macrophages disappeared at constant rates over the entire observation period. After the first week of exposure, the disappearance rates of small and medium peritoneal lymphocytes were identical. This rate was greater than that for peritoneal macrophages and that for circulating lymphocytes.

Based on the fraction of cells surviving any given exposure, the mononuclear leucocytes may be arranged in the following order of decreasing sensitivity to continuous low dose rate gamma irradiation: circulating lymphocytes, small peritoneal lymphocytes, medium peritoneal lymphocytes, peritoneal macrophages. This order is the same as that after acute exposure to X rays.

NON-TECHNICAL SUMMARY

The Problem

Prolonged continuous irradiation at low dose rates can impair hematopoiesis, increase susceptibility to infections, inhibit the immune response and shorten the life span of experimental animals. Civilian and military personnel exposed to radiation may be expected to suffer similar injuries. Therefore, it would be important to understand more precisely the effects of continuous low dose rate exposure so as to be able to evaluate the hazards of a fall-out field, of possible clandestine radioactive materials, or of having to accomplish a task while exposed to low levels of radiation for various time periods.

As part of an effort to elucidate the mechanisms of increased susceptibility to infection and of impairment of the immune response after irradiation, we initiated a study of the peritoneal leucocytes of mice and the effects of acute and chronic irradiation on this cell population. We have already reported that the peritoneal cells of LAF_1 mice decreased in number following a single whole-body exposure to X rays, that this effect was dose dependent, and that the different types of mononuclear cells present in the peritoneal cavity differed in their response to irradiation.

The present report describes the changes in the number of free cells in the peritoneal cavity and of lymphocytes in the circulating

blood during continuous exposure of mice to gamma radiations from a Co^{60} source at a dose rate of 1.4 rads per hour.

The Findings

During the first week of irradiation (accumulation of 190 rads) the lymphocytes in the circulating blood and the small lymphocytes in the peritoneal cavity disappeared at more rapid rates than during the subsequent 14 weeks (maximum accumulation 3450 rads). Medium peritoneal lymphocytes and peritoneal macrophages disappeared at constant rates over the entire observation period. After the first week of exposure, the disappearance rates of small and medium peritoneal lymphocytes were the same. This rate was greater than that for circulating lymphocytes and that for peritoneal macrophages.

Based on the fraction of cells surviving any given exposure, the mononuclear leucocytes in the peritoneal cavity and in the blood stream may be arranged in the following order of decreasing sensitivity to continuous low dose rate gamma irradiation: circulating lymphocytes, small peritoneal lymphocytes, medium peritoneal lymphocytes, peritoneal macrophages. This order is the same as that after acute X irradiation.

INTRODUCTION

A single acute exposure to ionizing radiations as well as prolonged continuous irradiation at low dose rates can impair hematopoiesis, increase susceptibility to infections, inhibit the immune response and shorten the life span of experimental animals. However, animals exposed to chronic irradiation must accumulate much larger doses than acutely irradiated animals before damage of similar extent can be detected (1-7).

The hematopoietic system is very sensitive to ionizing radiation. An extensive literature exists on the effects of acute exposure, but relatively little systematic information is available on the responses of experimental animals subjected to continuous irradiation at low dose rates. Several workers found a decrease in peripheral leucocyte counts of mice and rats exposed to gamma radiations for prolonged periods (1, 3, 8, 9, 10). Spargo and coworkers (11) observed a progressive depletion of the hematopoietic organs of mice receiving 4.4 or 8.8 rads daily. Lamerton, et al. (10) and Lord (12) reported that the cellularity of the bone marrow of rats given 50 or 84 rads per day decreased rapidly early in the irradiation period. After the third week of exposure, the nucleated cell counts either decreased more gradually or seemed to level off.

The free cells in the peritoneal cavity constitute another population of leucocytes which may be studied experimentally. In our previous reports we presented data showing that the peritoneal cells of LAF_1 mice decreased in number following a single total-body exposure to X rays, that this decrease was dose dependent, and that the different types of mononuclear cells present in the peritoneal cavity differed in their response to irradiation (13, 14). The present report is concerned with the effects of continuous exposure of LAF_1 mice to γ radiations from a Co^{60} source at a dose rate of 1.4 rads per hour.

MATERIALS AND METHODS

Mice

Female LAF_1 (C57L ♀ x A/He ♂) mice from our laboratory colony were used in all experiments.

Irradiation

Plastic mouse cages housing 10 mice each were placed on curved wooden racks so that the center of each cage was equidistant from a lead-shielded 10.8 curie Co^{60} source. Dose measurements, made with TLD LiF thermoluminescent dosimeters, indicated a dose rate of 1.4 rads per hour. The Co^{60} source was in continuous operation except for 1 hour per week when clean cages, food pellets and fresh drinking water were supplied.

Mice were irradiated continuously until accumulation of the desired dose. Length of exposure ranged from 6 to 103 days, accumulated doses from 190 to 3435 rads. All cell counts were made immediately after

removal of the animals from the exposure chamber.

In experiment I, all mice, with the exception of one group, were 13-16 weeks of age when irradiation was terminated and cell counts were made. Consequently, radiation exposure was begun at various ages. The youngest mice to be exposed were 4 weeks old. In experiment II, all mice were 8 weeks old when they were placed into the radiation chamber, their age at sacrifice varied from 9-18 weeks.

Control mice of each age group were held without exposure to irradiation and sacrificed at the same time as their irradiated partners. No deaths occurred during the irradiation period.

Blood counts

Blood was obtained from the tail of each mouse and diluted in 3% acetic acid containing a trace of crystal violet. Total leucocytes and lymphocytes (i.e., mononuclear cells) were counted in a hemacytometer. The values for individual mice in each group were averaged. All specimens were collected at approximately the same time of day (mid-morning).

Peritoneal cell counts

After the mice had been bled they were killed by cervical dislocation and the free cells in the peritoneal cavity were washed out with Tyrode's solution. The washings from each group were pooled. Leucocytes were counted in a hemacytometer. The total number of leucocytes harvested per mouse was calculated from the hemacytometer count and the volume of cell suspension collected.

Differential counts were made under phase contrast. At least 500 cells were counted from each preparation. Macrophages and lymphocytes were distinguished on the basis of morphological characteristics described previously (15). Lymphocytes were classified also according to size as either small (6 μ in diameter) or medium (8-10 μ in diameter). The number of macrophages and of small and medium lymphocytes per mouse was calculated from the percentage of each cell type present and the total cell count.

Statistical calculations

Regression and correlation coefficients were calculated by a computer program based on the method of least squares.

RESULTS

Table I lists the counts of circulating lymphocytes, small and medium peritoneal lymphocytes and peritoneal macrophages of control mice of various ages. Differential counts of the peritoneal cells are also shown. Mean cell counts of control mice aged 13-16 weeks (experiment I) and of mice aged 9-18 weeks (experiment II) did not differ statistically ($p > 0.05$). Standard deviations of the mean cell counts were not greater for the group of wider age span. Consistent changes in cell counts with increasing age could not be detected in the controls for experiment II. The regression coefficient of total peritoneal cell counts on age, using all the control mice shown in the table, did not differ significantly from 0 ($p > 0.05$). It was concluded, therefore, that the techniques used in the present experiments were not sensitive enough to detect any

TABLE I

CIRCULATING AND PERITONEAL MONONUCLEAR LEUCOCYTE COUNTS OF CONTROL MICE

Group	No. of mice	Age (weeks)	Circulating lymphs*	Peritoneal cells**			Differential counts (peritoneal cells)		
				Small lymphs	Medium lymphs	Macro-phages	Small lymphs	Medium lymphs	Macro-phages
							%	%	%
I A,C	5	15	----	19.3	28.2	26.7	26	38	36
B	5	14	7760	13.3	19.7	20.2	25	37	38
D	5	13	----	13.5	30.8	19.9	21	48	31
D	5	13	7010	9.7	26.4	17.8	18	49	33
E	5	14	11140	7.7	24.8	15.8	16	51	33
F	5	13	8430	9.2	43.7	23.8	12	57	31
G	5	14	5310	14.9	37.2	22.3	20	50	30
H	5	16	6840	10.5	27.3	14.7	20	52	28
J	8	20	9375	14.2	36.2	20.5	20	51	29
II A	5	9	8150	8.1	26.1	20.1	15	48	37
A	5	9	8650	8.6	19.5	19.5	18	41	41
B	5	11	8720	10.4	32.5	18.4	17	53	30
B	5	11	6450	13.8	29.2	14.4	24	51	25
C	5	13	6250	15.3	33.3	18.0	23	50	27
C	5	13	7250	13.1	27.9	18.4	22	47	31
D	5	15	6690	11.6	28.9	17.3	20	50	30
D	5	15	5430	19.1	34.6	19.9	26	47	27
E	5	18	8070	10.8	49.5	17.1	14	64	22
E	4	18	7550	8.9	31.9	15.1	16	57	27
Means ± 95% confidence limits									
I A-H	40	13-16	7750 ± 2060	12.3 ± 3.2	29.8 ± 2.7	20.2 ± 3.4	19.8	47.8	32.5
II A-E	49	9-18	7320 ± 790	12.0 ± 2.4	31.3 ± 5.5	17.8 ± 1.4	19.5	50.8	29.7
All	97	9-20	7590 ± 750	12.2 ± 1.6	30.9 ± 3.6	18.9 ± 1.9	19.6	49.5	30.8

*Number of cells per mm^3 blood
**Number of cells x 10^5 per mouse

changes in peritoneal cell counts with increasing age, at least over the 9-20 week age span. Consequently, the control values shown in Table II are those calculated from data for all the unirradiated animals employed in this study.

The cell counts of mice exposed continuously to γ radiation at a dose rate of 1.4 rads per hour are given in Table II. These cell counts are expressed also as percentages of the corresponding control values. Essentially no differences are detectable between experiments I and II. If the percentages of surviving cells at any one accumulated dose are compared, the mononuclear leucocytes of LAF_1 mice may be arranged in the following order of decreasing sensitivity to continuous irradiation: circulating lymphocytes, small peritoneal lymphocytes, medium peritoneal lymphocytes, peritoneal macrophages.

The cell counts of continuously irradiated mice are shown also in Figure 1. Each experimental point represents 5 mice; each control point is the mean cell count and 95% confidence interval of 97 mice. Inspection of the figure reveals that the disappearance curves of circulating lymphocytes and small peritoneal lymphocytes are biphasic. These cells disappeared at more rapid rates during the first week of exposure than during subsequent weeks. Therefore, disappearance rates of circulating lymphocytes and small peritoneal lymphocytes were calculated from cell counts of irradiated mice only. In contrast, medium peritoneal lymphocytes and peritoneal macrophages disappeared at uniform rates over the entire exposure period. Disappearance rates of these cells were calculated

TABLE II

CIRCULATING AND PERITONEAL MONONUCLEAR LEUCOCYTE COUNTS OF CONTINUOUSLY IRRADIATED MICE (1.4 RADS/HOUR)

Group	Total dose (rads)	No. of mice	Circulating lymphs*	Peritoneal Cells**			Circulating lymphs	Peritoneal Cells		
				Small lymphs	Medium lymphs	Macrophages		Small lymphs	Medium lymphs	Macrophages
							Percent of control			
Control	0	97	7590	12.2	30.9	18.9	100	100	100	100
I A	190	10	870	7.2	24.0	22.8	11.5	59.0	77.7	120.6
B	435	10	----	7.5	16.9	14.2	----	61.5	54.7	75.1
C	660	10	1040	5.5	22.3	16.9	13.7	45.1	72.2	89.4
D	1130	10	830	2.4	15.5	14.1	10.9	19.7	50.2	74.6
E	1560	10	600	3.2	15.8	12.2	7.9	26.2	51.1	64.5
F	1790	7	510	1.0	5.7	7.5	6.7	8.2	18.4	39.7
G	2895	10	480	0.7	4.7	6.9	6.3	5.7	15.2	36.5
H	2765	10	590	1.3	3.8	7.6	7.8	10.6	12.3	40.2
J	3435	8	530	1.0	2.6	6.0	7.0	8.2	8.4	31.7
II A	190	10	1705	6.0	19.3	17.7	22.5	49.2	62.4	93.6
B	695	10	1100	3.5	13.9	14.3	14.5	28.7	45.0	75.7
C	1095	10	1130	3.2	9.0	11.2	14.9	26.2	29.1	59.2
D	1630	10	1080	2.1	7.7	7.1	14.2	17.2	24.9	37.6
E	2295	10	695	1.5	8.3	6.4	9.1	12.3	26.9	33.9

*Number of cells per mm^3 blood
**Number of cells x 10^5 per mouse

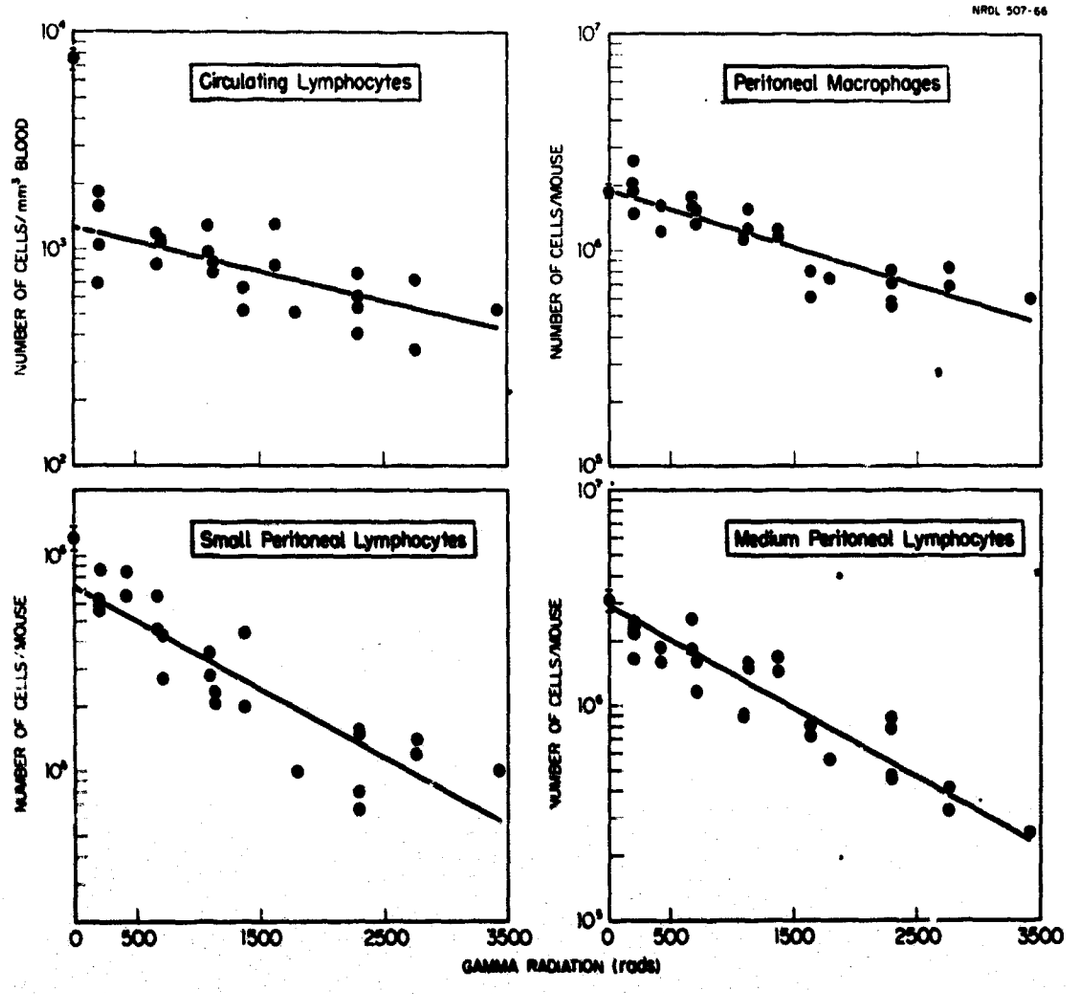

Figure 1. Effect of continuous gamma irradiation of LAF_1 mice on counts of circulating and peritoneal mononuclear leucocytes.

from data of irradiated and control mice.

The question was raised whether the disappearance curve of small peritoneal lymphocytes should be considered as biphasic. On the assumption that the small peritoneal cells disappeared at a constant rate during the entire irradiation period, the disappearance rate was calculated also from cell counts of irradiated and control mice. The resulting regression line had a slope of -0.394×10^{-3} and intercepted the origin at a point below the 95% confidence limit of the observed mean control value, in spite of the relatively great weight of the control data in the calculation. For this reason, the disappearance curve of small peritoneal lymphocytes was interpreted as being biphasic and the disappearance rate was calculated from data of irradiated mice only.

The disappearance rates (regression coefficients of cell counts on accumulated dose) are presented in Table III. It is interesting to note that the disappearance rates of small and medium peritoneal lymphocytes were identical. However, peritoneal lymphocytes disappeared at a more rapid rate than circulating lymphocytes.

DISCUSSION

Continuous exposure of LAF_1 mice to γ radiation at a dose rate of 1.4 rads per hour reduced the number of mononuclear leucocytes in the circulating blood and in the peritoneal cavity. However, the kinetics of disappearance differed for various cell types. The small lymphocytes in the peritoneal cavity and in the circulating blood had biphasic

TABLE III

REGRESSION AND CORRELATION COEFFICIENTS OF MONONUCLEAR CELL COUNTS ON ACCUMULATED RADIATION DOSE

	Regression coefficient ± 95% confidence limit	Correlation coefficient
	x 10^{-3}	
Circulating lymphocytes	-0.137 ± 0.063	0.691
Peritoneal cells		
Small lymphocytes	-0.315 ± 0.068	0.889
Medium lymphocytes	-0.310 ± 0.034	0.938
Macrophages	-0.176 ± 0.025	0.900

disappearance curves. These cells disappeared more rapidly during the first week of irradiation than during subsequent weeks. In contrast, medium lymphocytes and macrophages in the peritoneal cavity disappeared at uniform rates over the entire observation period.

The early rapid decreases in populations of small lymphocytes may be attributed largely to the lethal action of low doses of γ radiation on these cells. The mononuclear cells in the circulating blood are primarily small lymphocytes (6 μ in diameter) which are indistinguishable under the light microscope from the small lymphocytes in the peritoneal cavity. Yet, during the first few days of γ irradiation, a much greater fraction of small lymphocytes disappeared from the blood stream than from the peritoneal cavity. The reasons for this finding may be several. In spite of their morphological similarity, the small lymphocytes in the circulation and in the peritoneal cavity may differ physiologically. Environmental differences may affect their sensitivity to irradiation. Damaged cells may be removed more effectively from the blood stream than from the peritoneal cavity. Recirculation routes of small lymphocytes may favor the peritoneal cavity over the peripheral blood.

The gradual reductions in cell counts over longer time periods are considered to be due to the interaction of the following and perhaps additional factors: life span of the cells, extent of damage (both immediate and latent) to mature cells and their precursors, possible alterations in generation time and recirculation patterns of the cells, rate of recovery. The sum of these factors, as they apply to each cell

type, is expressed by the disappearance rate. It is interesting to note that this rate is greater for lymphocytes in the peritoneal cavity than for those in the circulation, and that the rates for small and medium peritoneal cells are identical (Table III). It is not possible to determine from the data available whether the latter observation is fortuituous or provides evidence for identical behavior of these cell populations after the first week of continuous low dose rate irradiation. Perhaps similar experiments at different dose rates could supply such information.

Spargo and corworkers (11) and Sacher (8) also found that the disappearance curves of circulating lymphocytes in continuously irradiated LAF_1 mice were biphasic. However, Hammond, et al.(3), who studied CF-1 mice, obtained relatively straight disappearance curves, although some reduction in the rate of decline was evident after the third week of exposure to 34 or 69 rads per day.

In contrast to the investigators who reported that blood counts of continuously irradiated mice decreased progressively, Lamerton and associates (10), using rats, found that after an initial rapid disappearance of lymphocytes from the circulating blood, a steady state was established during which the cell counts remained essentially unchanged in spite of continued exposure to gamma rays at dose rates of 16, 50, or 84 rads per day.

Species and strain differences in experimental animals may account for the different lymphocyte disappearance curves obtained by the

investigators cited. On the other hand, the present experiments show that in the same strain of mice, morphologically distinct mononuclear leucocytes differed in disappearance kinetics during continuous irradiation. In all instances, however, the cell counts declined progressively. No evidence was obtained for the establishment of a steady state 2-3 weeks after exposure.

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13. ABSTRACT

Continuous exposure to Co^{60} γ irradiation delivered at 1.0-1.5 rad/hour increases the susceptibility of mice to subcutaneous and airborne infections with strains of Listeria monocytogenes and Pasteurella tularensis of relatively low virulence for non-irradiated mice.

Although the irradiated mice were found to be fully as capable as non-irradiated mice of synthesizing antibodies against sheep red blood cells and of rejecting foreign skin grafts, survivors of an initial infection were less resistant to subsequent infection than non-irradiated mice. Data previously reported, together with the data from these experiments, suggest that macrophages of irradiated animals are readily injured by bacteria or their products. Hence, even immune macrophages may be unable to effectively destroy the invading bacteria.

Preliminary results indicated that the protective effect of WR-1607, a radioprotective chemical may be abolished by subsequent infection with Pasteurella tularensis.

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